

WHAT IS CLAIMED IS:

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1. A process for preparing recombinant adenovirus, the process comprising:
- (a) preparing a culture of producer cells in a selected media; • •
- (b) infecting producer cells in the culture with recombinant adenovirus, wherein the producer cells are infected between mid-log phase of growth and stationary phase of growth; and
- 10 (c) harvesting recombinant adenovirus from the cell culture.
- 15 2. The process of claim 1, wherein the producer cells are infected with the adenovirus between late-log phase and stationary phase of growth.
3. The process of claim 1, wherein the producer cells are seeded using an essentially homogeneous pool of cells.
- 20 4. The process of claim 1, wherein the producer cells are perfused for at least a portion of the time that the cells are cultured.
5. The process of claim 4, wherein the producer cells are perfused at a rate that will maintain a glucose level of between about 0.5 and about 3.0 gm glucose/liter.
- 25 6. The process of claim 5, wherein the producer cells are perfused at a rate that will maintain a glucose level of between about 0.7 and about 2.0 gm glucose/liter.
7. The process of claim 6, wherein the producer cells are perfused at a rate that
- 30 maintains a glucose level of between about 1 and about 1.5 gm glucose/liter.

8. The process of claim 1, wherein the producer cells are seeded into the culture medium and allowed to attach to a culture surface for between about 3 hours and about 24 hours prior to infection with adenovirus.

9. The process of claim 1, wherein the culture medium is at least partially recirculated during the adenovirus infection step.

10. The process of claim 1, wherein the culture medium is seeded with between about 0.5×10^4 and about 3×10^4 cells/cm².

11. The process of claim 10, wherein the culture medium is seeded with between about 7.5×10^3 and about 2.0×10^4 cell/cm².

12. The process of claim 11, wherein the culture medium is seeded with between about 9×10^3 and 1.5×10^4 cells/cm².

13. The process of claim 1, wherein the harvested adenovirus is subjected to purification and placed into a pharmaceutically acceptable composition.

14. The process of claim 13, the adenovirus is purified by steps which include chromatography.

15. The process of claim 14, wherein the chromatography step involves subjecting the adenovirus to more than one chromatographic separations.

16. The process of claim 14, wherein the chromatography step involves subjecting the adenovirus to only one chromatographic separation.

17. The process of claim 16, wherein the chromatographic separation includes ion-exchange chromatography.

18. The process of claim 1, wherein said recombinant adenovirus is a replication-deficient adenovirus encoding a therapeutic gene operably linked to a promoter.

19. The process of claim 18, wherein said replication deficient adenovirus is lacking at least a portion of the E1 region.

20. The process of claim 19, wherein said producer cells complement the growth of replication deficient adenovirus.

21. The process of claim 1, wherein said producer cells are selected from the group consisting of 293, PER.C6, 911 and IT293SF cells.

22. The process of claim 21, wherein said producer cells are 293 cells.

23. The process of claim 1, wherein said recombinant gene is selected from the group consisting of antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl* antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, *zac1*, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, mda-7, thymidine kinase or p53.

24. The process of claim 23, wherein said recombinant gene is a p53 gene.

25. The process of claim 1, wherein said promoter is an SV40 IE, RSV LTR, β -actin, CMV-IE, adenovirus major late, polyoma F9-1, or tyrosinase promoter.

26. The process of claim 1, wherein the adenovirus is harvested by steps that include lysing the producer cells by means other than freeze-thaw.

5 27. The process of claim 26, wherein the producer cells are lysed by means of a detergent lysis.

28. The process of claim 26, wherein the producer cells are lysed by means of autolysis.

10 29. The process of claim 1, further comprising purifying the harvested adenovirus to obtain a purified recombinant adenovirus composition having one or more of the following properties:

15 (a) a virus titer of between about 1×10^9 and about 1×10^{13} pfu/ml;

(b) a virus particle concentration between about 1×10^{10} and about 2×10^{13} particles/ml;

20 (c) a particle:pfu ratio between about 10 and about 60;

(d) having less than 50 ng BSA per 1×10^{12} viral particles;

25 (e) between about 50 pg and 1 ng of contaminating human DNA per 1×10^{12} viral particles,

(f) a single HPLC elution peak consisting essentially of 97 to 99% of the area under the peak.

30. A recombinant adenovirus composition comprising between 5×10^{14} and 1×10^{18} viral particles, prepared by a process in accordance with claim 1.

✓ 31. A purified recombinant adenovirus composition comprising between 5×10^{14} and 1×10^{18} adenoviral particles, said composition having one or more of the following properties:

- (a) a virus titer of between about 1×10^9 and about 1×10^{13} pfu/ml;
- 10 (b) a virus particle concentration between about 1×10^{10} and about 2×10^{13} particles/ml;
- (c) a particle:pfu ratio between about 10 and about 60;
- 15 (d) having less than 50 ng BSA per 1×10^{12} viral particles;
- (e) between about 50 pg and 1 ng of contaminating human DNA per 1×10^{12} viral particles,
- 20 (f) elutes essentially as a single elution peak upon HPLC.

32. The composition of claim 31, wherein the composition has a viral titer of between about 1×10^{11} and about 1×10^{13} pfu/ml.

25 33. The composition of claim 32, wherein the composition has a viral titer of between about 1×10^{12} and about 1×10^{13} pfu/ml.

34. The composition of claim 31, wherein the composition has a virus particle concentration between about 1×10^{11} and about 2×10^{13} .

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35. The composition of claim 34, wherein the composition has a virus particle concentration between about 1×10^{12} and about 1×10^{13} .

5 36. The composition of claim 31, wherein the composition has a particle:pfu ratio between about 10 and about 50.

37. The composition of claim 36, wherein the composition has a particle:pfu ratio between about 10 and about 40.

10 38. The composition of claim 37, wherein the composition has a particle:pfu ratio between about 20 and about 40.

15 39. The composition of claim 31, wherein the composition has between about 1 ng and 50 ng BSA per 1×10^{12} viral particles.

40. The composition of claim 39, wherein the composition has between about 5 ng and 40 ng BSA per 1×10^{12} viral particles.

20 41. The composition of claim 31, wherein the composition has between about 50 pg and 500 pg of contaminating human DNA per 1×10^{12} viral particles.

42. The composition of claim 41, wherein the composition has between about 100 pg and 500 pg of contaminating human DNA per 1×10^{12} viral particles.

25 43. The composition of claim 31, wherein the adenovirus of said composition elutes as essentially a single HPLC peak that comprises between 97 and 99% of the total area under the peak.